Research training report

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Home lab: Laboratory for Experimental Neuroanatomy, Institute of Anatomy „Niko Miljanic“, School of Medicine, University of Belgrade
Host lab: Biology-Biochemistry lab, Faculty of Nursing, National and Kapodistrian University of Athens
Dates of training stay: 15/1/2018 – 2/3/2018

It is my pleasure to report the outcome of the research training completed at Biology-Biochemistry lab at NKUA. During the period between 15/1 – 2/3, I was trained in performing the following methods:

- Attention Set Shifting Task (ASST)
- Isolation of specific brain areas
- Western blot (WB)
- Sectioning on cryostat
- Immunostaining and Image analysis

ASST

For this purpose, 2 cohorts of animals were trained and tested in a non-transparent Plexiglas box, divided by the wooden board into a „waiting“ and „testing“ compartment. Following a 3 days partial food restriction, during the next 5 days animals were trained in digging the material found in a ceramic pot, where the food reward was placed. The training was successfully completed if an animal performed 10 correct digs. On day 6, simple rule acquisition was carried out, when animals were trained to discriminate between 2 different digging media, with one of them containing a food reward. Six consecutive correct trials was set as a reaching criterion for proceeding to the next phase. Next day, a compound discrimination task was applied afther reaching the criterion in simple discrimination task, as on the previous day. For this purpose, odor was introduced as an irrelevant stimulus, while digging medium persisted as a relevant one. After reaching the criterion, animals proceeded to the intra-dimensional set shift phase, when a new odor (irrelevant stimulus) substituted the previous one. In the next phase – intra-dimensional reversal shift, rewarded and non-rewarded pots containing different digging media as a relevant stimulus were switched. On day 8, animals were exposed to a compound discrimination, an intra-dimensional set shift and an intra-dimensional reversal shift, using novel digging materials and odors, with the type of digging material being again the predictor of reward. Following intra-dimensional reversal shift phase, an extra-dimensional shift trial was implemented with two novel odors now being the relevant stimulus and a single novel digging material being the irrelevant one. After the criterion was reached, a second intra-dimensional set shift and a second intra-dimensional reversal shift were performed respectively with odor as the relevant stimulus.

Isolation of specific areas and Western blot

In order to be trained in isolating specific brain areas we used rat pup brains and young adult rat brains provided by the host lab. I was trained in isolating the following brain areas: prefrontal cortex, ventral striatum, amygdala, hippocampus, somatosensory cortex and hypothalamus. Tissue homogenization was performed using RIPA buffer containing protease inhibitors, after which the proteins

were extracted following centrifugation. Total protein concentration was estimated using the Bradford assay. Western blot training included sample loading, SDS-PAGE gel running, gel to membrane protein transfer, blocking the non-specific binding, antibody incubation for M1 cholinergic receptors, ECL exposure and chemiluminescent signal detection. The first few trials were dedicated to observing and assisting in the procedure before performing these procedures independently. Signal detection was performed in the dark room using x-ray films. After obtaining the signal I was trained in quantifying the band density ratio and analyzing the results in ImageJ (Figure). I would like to emphasize the great amount of help provided by everyone in the laboratory. Everyday discussions regarding both practical and theoretical aspects of such touchy scientific procedure such as WB were crucial in gaining the ability to perform this method independently.

Sectioning on cryostat, immunostaining and Image analysis

Cryosectioning was performed on a freezing cryostat (Leica CM1900, Nussloch, Germany) using rat pup and adult rat brains previously flash-frozen in -40°C isopentane. I was trained in collecting serial coronal sections (20μm thick) on SuperFrost Plus glass slides in a standard, systematic sequence so that six sections 240μm apart were present on each slide. Slides were stored at -80°C. For immunostainings, sections were thawed at RT and post-fixed in 4% paraformaldehyde. Following washes in PBS-Triton X-100 we proceeded to the blocking of non-specific binding and primary antibody incubation. Depending on the antibody used (anti-vGLUT or/and anti-GAD67) 1-3 days of incubation were selected. The second day of immunostainings was performed using fluorochrome conjugated secondary antibodies, after which the slides were coverslipped using Mowiol mounting medium. After optimizing the antibody and reagents concentrations along with getting the satisfying signal I was trained in microscopic image capturing using the Infinite Capture v.6.0 (Lumenera Co., Ottawa, Canada) software and image analysis using ImageJ software. Significant attention was dedicated to understanding the principles of cell counting, the importance of adjusting the concentrations of antibodies and reagents as well as identifying brain areas according to the Paxinos and Watson rat brain atlas.

Future implementation and professional development

I am happy to attest that my research training at Biology-Biochemistry lab was a very fruitful and important experience. Beside the skills and methods I have learned, I expanded my knowledge in better understating the principles of early life stress along with the purpose of implementing particular scientific methods and their feasibility. I would also like to say that I enjoyed working in the scientific team of prof. Stamatakis and prof. Kitraki, a team that provided great working and learning atmosphere. It will be a great challenge for me and my colleagues to reach such environment in our laboratory in Belgrade and to continue the collaboration in years to come.

Soon after my return, we arranged the room at our institute that will be dedicated for future behavioral analysis and equipped it with the ASST box. In our experiment, we plan to use the Western blot method initially at the Institute of Biochemistry at Faculty of Medicine in Belgrade. Primarily, we will analyze the density of M1 and M2 receptors in the prefrontal cortex and amygdala in both rat pups and young adult rats along with the expression of Iba1 in the respected regions in the rat pups. Also, during 2018, we expect a new purchase of equipment for our laboratory needed for the performance of immunostainings, a method essential for our scientific work. I am looking forward to implementing my newly acquired knowledge and skills.

Finally, I would like to thank NENS for supporting not only my professional development, but also development of the Laboratory for Experimental Neuroanatomy by giving me the opportunity to complete the research training and continue the cooperation with our colleagues in Athens. We look forward to the upcoming challenges.

Sincerely,
Joko Poleksić

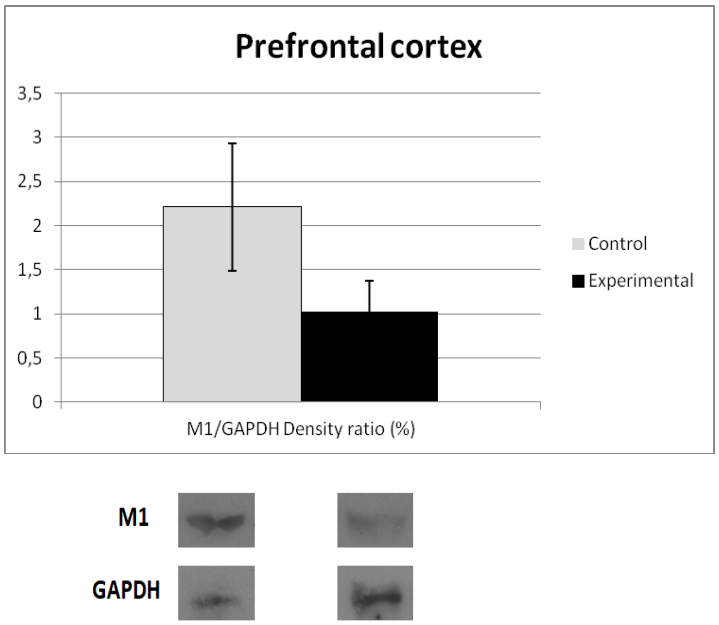

Figure. Bars represent means ± SEM of the relative optical density M1/GAPDH. No statistical significant difference were found in the prefrontal cortex. (Students' t-test for two independent groups, $p<0.05$).